Kidney International, Vol. 41 (1992), pp. 813–821

Relationships among natriuresis, atrial natriuretic peptide and insulin in insulin-dependent diabetes

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Relationships among natriuresis, atrial natriuretic peptide and insulin in insulin-dependent diabetes. Insulin-dependent diabetic patients have a large exchangeable body sodium pool, secondary to sodium retention. The pathogenesis of impaired natriuresis in insulin dependent diabetes remains to be elucidated. The present study examines the role of hyperinsulinemia, impaired atrial natriuretic release, and resistance to atrial natriuretic peptide action in determining sodium retention in normotensive and hypertensive insulin-dependent diabetic patients. Eight insulin-dependent diabetic patients had significantly higher daily sodium excretion rate (147 ± 16 mmol/day; mean ± SE) during conventional insulin treatment (daily plasma glucose: 11.6 ± 1.2 mmol/liter; daily plasma insulin: 27 ± 3 μU/ml) than during intensified insulin treatment (daily sodium excretion rate: 91 ± 12, $P < 0.01$; daily plasma glucose: 6.8 ± 0.7, $P < 0.01$; daily plasma insulin: 44 ± 4, $P < 0.01$). Daily sodium excretion rate was also significantly lower (107 ± 13, $P < 0.01$) in the same diabetic patients during intensified insulin treatment along with hyperglycemic clamp (daily plasma glucose: 12.8 ± 0.3, NS; plasma insulin 48 ± 4, $P < 0.01$). Seven control subjects had lower extracellular liquid volume than eight insulin-dependent diabetic patients (11.0 ± 0.8 l/ 1.73 m^2 vs. 14.8 ± 0.9, $P < 0.05$) and also had baseline plasma atrial natriuretic peptide concentrations (18 ± 5 pg/ml vs. 37 ± 4, $P < 0.05$). Atrial natriuretic peptide response to saline challenge was blunted in insulin-dependent diabetic patients when saline was administered on the basis of body surface area (90 mmol/1.73 m^2 · 90 min) but not when administered on the basis of extracellular liquid volume (ECV) (8.2 mmol/liter ECV 90 min). Continuous infusion of atrial natriuretic peptide in the same control and diabetic subjects (0.06 μg/kg · min · 120 min) resulted in similar circulating concentrations of the hormone but in significantly lower sodium excretion rate in diabetic (from 185 ± 19 to 193 ± 21 μmol/1.73 m^2 · min) than in control subjects (199 ± 14 to 341 ± 22, $P < 0.01$). Natriuretic action of atrial natriuretic peptide was similarly impaired in a group of eighteen hypertensive insulin-dependent diabetic patients in comparison with a matched group of seven hypertensive control patients. Angiotensin converting enzyme inhibitor treatment in these hypertensive diabetic patients decreased extracellular liquid volume and improved natriuretic response to atrial natriuretic peptide. We conclude that refractoriness to natriuretic action rather than impaired release of atrial natriuretic peptide can further deteriorate sodium retention in insulin dependent diabetes. This altered hormonal behavior could be primarily due to insulin-induced sodium retention and extracellular liquid volume expansion.

A large exchangeable body sodium pool has been reported in normotensive [1–3] and hypertensive [4] IDDM patients who are without evidence of renal or vascular complications. Exchangeable body sodium pool is further increased in IDDM patients with established nephropathy (defined as albumin excretion of greater than 500 mg/24 hr) [5] and also in those with microalbuminuria or incipient nephropathy (defined as albumin excretion rate between 30 and 500 mg/24 hr) [6].

The increased exchangeable mass of sodium is accompanied by a concomitant increase in the ECV [6, 7] and of plasma volume [8], although this latter finding has not been uniformly confirmed [4, 6]. An expansion in sodium exchangeable mass has been frequently advocated to explain the frequent association between hypertension and IDDM [9]. However, it has to be pointed out that some studies have also reported that the prevalence of hypertension in IDDM is similar to that showed by a non-diabetic population [10]. From a hemodynamic point of view, a larger plasma volume and an expanded ECV could lead to increased cardiac output and in turn to increased blood pressure levels and glomerular filtration rate. This cascade of events should be eventually able to buffer the expansion in the magnitude of ECV [11, 12]. According to this hypothesis, the expansion in plasma volume is an early and transient phenomenon which is rapidly overcome by the cardiovascular feedback system to maintain a stable hemodynamic condition in all tissues [11, 12], and it is therefore missed if plasma volume is evaluated when this hemodynamic rearrangement has already occurred.

Whatever the relationship between plasma and ECV, IDDM patients have impaired natriuretic response to volume expansion either induced by head-out water immersion [5, 13, 14] or by saline infusion [3]. The pathogenesis of this impaired capacity of handling a sodium load in IDDM is unclear. The present study examines the role of insulin and ANP in determining sodium retention in IDDM.

Methods

Patients

Twenty-six type I, insulin-dependent diabetic (IDDM) patients with onset of the disease before the age of 30, aged 17 to 42 years, were divided into two groups: normotensives ($N = 8$) with blood pressure levels below 140 and 85 mm Hg and hypertensives ($N = 18$) with blood pressure levels above 145 and 90 mm Hg, for systolic and diastolic values, respectively,

according to the statement on Hypertension in Diabetes Mellitus [15]. Seven non-diabetic hypertensive patients and seven normotensive healthy subjects were selected from those attending the outpatient clinic of the Internal Medicine Department, University of Padova. A complete medical workup was performed to rule out the diagnosis of secondary hypertension. Kidney, liver and endocrine (except for IDDM) function was normal. An additional criterion for inclusion in the study was the presence of normal body weight (<15% above or below desirable body weight) [16]. IDDM patients with duration of diabetes higher than 11 years were not included. Patients with neovascularization and fibroproliferation on standard eye examination, or with autonomic neuropathy, were also not taken into consideration. All IDDM patients were taking two or three daily subcutaneous insulin injections. Only patients who showed HbA_{1c} values lower than 7.5% in the 12 months preceding the study were included. In the six months preceding the study, each patient was visited at least twice a month by one of the physicians involved in the study. Major efforts were dedicated to improve the degree of metabolic control. Each patient was admitted to the hospital one week before the study. Eight out of the eighteen hypertensive IDDM patients were on antihypertensive therapy: five on beta-blockers, two on calcium antagonists and one on angiotensin converting enzyme inhibitors. Hypertensive IDDM patients on antihypertensive treatment had sodium excretion rates not significantly different from those of patients who were without previous treatment during the week of admission in the ward. Any antihypertensive therapy was discontinued one week before hospital admission and during hospital stay, and subjects followed an isocaloric diet containing 50% carbohydrate, 25% fat, and 25% protein. The daily intake of NaCl was 100 mmol/day during the week preceding each study. All control and IDDM subjects were considered to be in sodium balance in the three days preceding each study on the basis of sodium intake and sodium excretion rate values. The estimated sodium intake before hospital admission was 100 to 150 mmol/day without significant differences between control and IDDM subjects. Plasma creatinine concentration was normal in all IDDM and control subjects. All subjects and patients had sterile urine and normal urine microscopy. The level of albumin excretion rate was determined as the median value of three 24-hour urine collections, free of ketone bodies within a fortnight. Control subjects and normotensive diabetic patients had an albumin excretion rate lower than 20 μg/min; 6 out of the 18 hypertensive diabetic patients had an albumin excretion rate higher than 20 μg/min and lower than 36 μg/min (Table 1). All subjects gave informed consent and the experimental design was approved by the local Ethical Committee of the University of Padova.

Table 1. Clinical features of normotensive (NC) and hypertensive (HC) control subjects normotensive (NP-IDDM) and hypertensive (HP-IDDM) insulin-dependent diabetic patients

	Control subjects		Diabetic patients	
	NC	HC	(NP-IDDM)	(HP-IDDM)
Number and gender	4M/3F	3M/4F	5M/3F	10M/8F
Body mass index *kg/m²*	23.9 ± 1.0	24.0 ± 1.1	22.9 ± 0.7	23.6 ± 0.8
Age *years*	33 ± 2	32 ± 2	31 ± 3	39 ± 4
Duration of diabetes *years*	—	—	10 ± 3	16 ± 4
Blood pressure S	122 ± 5	160 ± 6	119 ± 4	152 ± 8
levels *mm Hg* D	77 ± 3	95 ± 5	76 ± 2	97 ± 3
Plasma renin activity *ng/ml/hr*	1.7 ± 0.2	1.9 ± 0.3	0.8 ± 0.2	0.9 ± 0.3
Plasma creatinine *μmol/liter*	88 ± 7	95 ± 7	80 ± 8	107 ± 11
Albumin excretion rate *μg/min*	5 (3–10)	12 (4–25)	8 (6–12)	18 (7–36)

Values are expressed as mean ± SE or as median with range. Abbreviations are: S, systolic; D, diastolic.

Procedures

Study 1: Effects of hyperinsulinemia. Normotensive IDDM patients were studied thrice: A) during conventional insulin regimen, with two daily injections of soluble (Actrapid HM, Novo, Rome, Italy) and long acting (Protophane HM, Novo) insulin preparations before breakfast and dinner (0.5 U/kg/day); B) during intensified insulin regimen with three daily injections of soluble insulin before breakfast, lunch and snack, and with one evening injection of soluble and long-acting insulin preparation before dinner (0.7 U/kg/day); C) during intensified insulin regimen as described in (B), but with variable amounts of glucose infusion in order to clamp blood glucose concentrations at hyperglycemic levels (between 10 and 20 mmol/liter), using a Beckman glucose analyzer to determine blood glucose concentration with a glucose-oxidase technique (Beckman, Milano, Italy) every fifteen minutes, and a Harvard continuous infusion pump (Harvard, Apparatus, Boston, Massachusetts, USA). Other details have been provided elsewhere [17]. Intermittent blood samples were withdrawn every 60 minutes to measure plasma glucose and free insulin concentrations. Twenty-four-hour urines were collected to determine daily sodium excretion rate. During protocol, an overnight fasting blood sample was taken for measurement of plasma renin and creatinine concentration.

Study 2: Atrial natriuretic peptide response to isotonic volume expansion. Normotensive IDDM patients and control subjects received a single intravenous injection of 3.7 mEq ^{51}chromium edetic acid (^{51}Cr EDTA) after an overnight fast at euglycemic levels achieved in IDDM patients by a subcutaneous overnight continuous infusion of insulin (Actrapid HM 15 mU/kg · hr with a bolus MC 20 insulin-infusion device; Miles, Ames, Cavenago, Milan, Italy) in order to measure ECV. Blood samples for tracer determination were drawn before and 1, 2, 3, 4, 5, 7, 10, 12.5, 15, 30, 45, 60, 90, 120, 150, 180, 210, 250, 300, 330, 360, 420 minutes after the bolus intravenous injection of ^{51}Cr EDTA. The time-course decay of tracer concentration followed a two exponential equation fit [18]. Both IDDM patients and control subjects received an intravenous infusion of 90 mmol/1.73 m² · 90 min (154 mM) (Saline I). Normotensive IDDM patients received an intravenous infusion of saline in a second occasion following the same procedures, except for the fact that saline load was administered of the basis of ECV, that is, 8.2 mmol/liter of ECV equal to that used in normotensive control subjects (that is, 8.2 mmol/liter of ECV) (Saline II). This latter saline load, however, was greater than that used in normal control subjects, when expressed as mmol/1.73 m² body surface area (121 vs. 90 mmol/1.73 m²), as IDDM patients had similar body surface area but larger ECV. Renal hemodynamics,

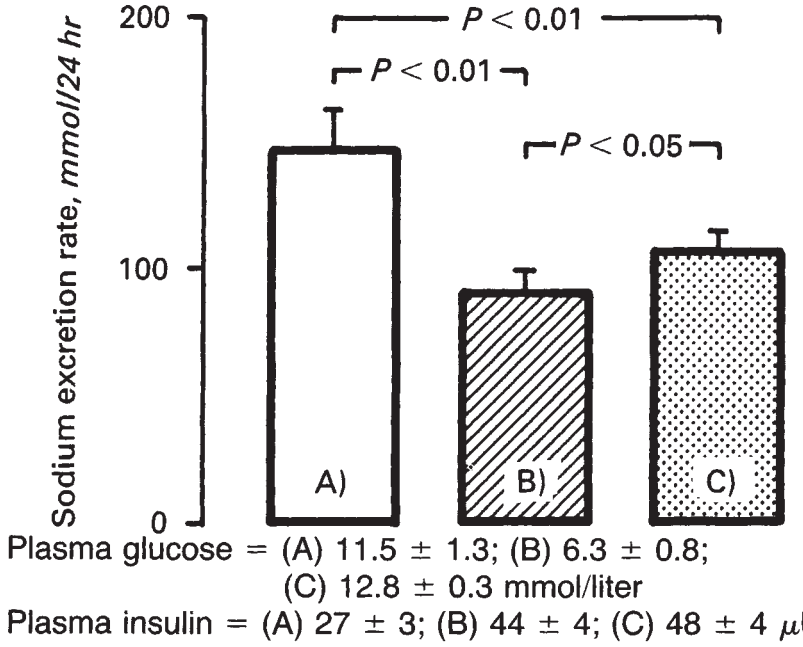

Fig. 1. *Mean ± values of daily sodium excretion rate during conventional* (**A**), *intensified* (**B**) *and intensified-hyperglycemic* (**C**) *insulin regimens in eight normotensive insulin-dependent diabetic patients.* Details are in the text.

hormonal parameters and sodium excretion rate were evaluated as described elsewhere [3, 19–21].

Study 3: Responsiveness to atrial natriuretic peptide. Renal and systemic responses to atrial natriuretic peptide (ANP) infusion were studied in seven normotensive and seven hypertensive non-diabetic patients, and eight normotensive and eighteen hypertensive diabetic patients. In addition the eighteen hypertensive diabetic patients were also studied in a second occasion after three months of treatment with angiotensin converting enzyme inhibitor (Cilazapril, Ro 31-2848, F. Hoffmann-La Roche Co. Ltd., Basel, Switzerland). All subjects were studied in the morning after an overnight fast in a recumbent position. Intravenous catheters were placed bilaterally for administration of ANP and blood sampling, respectively. Urine samples were obtained by free voiding at timed intervals. After a stabilization period of 30 to 60 minutes following intravenous catheter placement, all individuals received an oral water load of 500 ml. Renal plasma flow and glomerular filtration rate were determined as previously described during Study 2. After completion of the 120-minute equilibration period, there were three 30-minute phases of urine collection at baseline, and four 30-minute phases of urine collection during the ANP constant infusion at a rate of 0.06 μg/kg · min · 120 min, preceded by a priming dose of 10 and 15 μg ANP bolus intravenous injection in non-diabetic and IDDM subjects, respectively. Urine output was replaced on a milliliter per milliliter basis throughout the study by administering oral water. All infusion were given by a constant-rate infusion pump, with the final concentration of ANP adjusted for patient's body weight. The administered volume during the experimental infusion period was 50 ml/1.73 m^2 · 120 min. Equal volumes of 0.9% sodium chloride were given during the baseline phase for each individual. Heart rate and cuff blood pressure were recorded at 10 minute intervals, throughout the study. Upon completion of lead-in stabilization and each of the urine collection periods, blood samples were obtained for ^{125}I-para-aminohippurate (^{125}I-PAH) and ^{51}Cr-EDTA radioactivity (dpm/ml). Serum electrolytes, hematocrit, serum protein (albumin and total) and plasma immunoreactive (ir) ANP were obtained upon completion of lead-in stabilization and each of the urine collection periods. Urine collections were obtained at time 0 and at the end of each collection period for volume, ^{125}I-PAH and ^{51}Cr-EDTA radioactivity (dpm/ml), and sodium. Plasma glucose, insulin, atrial natriuretic peptide, hemoglobin A_{1c}, electrolyte and protein concentrations were measured as described in detail elsewhere [3, 19–21], as well as glomerular filtration rate, renal plasma flow, sodium excretion and albumin excretion rate [3, 19–21]. Standard parametric and non-parametric statistical analysis was used to assess the degree of significant differences [3, 19–21].

Results

Effects of hyperinsulinemia with euglycemia (Study 1)

IDDM patients, during conventional insulin treatment, had a daily mean value of plasma glucose of 11.5 ± 1.3 (mmol/liter) and plasma insulin of 27 ± 3 (μU/ml). During intensified insulin regimen with strict metabolic control plasma glucose concentration decreased to 6.3 ± 0.8 (mmol/liter; $P < 0.01$) and plasma insulin concentration rose to 44 ± 4 (μU/ml; $P < 0.01$). During intensified insulin regimen along with hyperglycemic glucose clamp plasma glucose concentration was between 12.5 and 15.0 mmol/liter throughout most of the day, whereas plasma insulin concentration was not significantly different from that found during intensified insulin regimen (48 ± 4 μU/ml) with strict metabolic control without additional glucose administration. Daily sodium excretion rate was 147 ± 16 mmol/24 hr during conventional insulin regimen (Fig. 1A) and decreased to 91 ± 12 mmol/24 hr ($P < 0.01$) during intensified insulin regimen with strict metabolic control (Fig. 1B) and to 107 ± 13 mmol/24 hr ($P < 0.01$) during the intensified insulin regimen along with hyperglycemic glucose clamp (Fig. 1C). Daily sodium excretion rate

during intensified insulin regimen with strict metabolic control was slightly but significantly lower than that during intensified insulin regimen along with hyperglycemic glucose clamp (Fig. 1 B vs. C, $P < 0.05$).

ANP response to isotonic volume expansion (Study 2)

ECV was larger in IDDM patients (14.8 ± 0.9 l/1.73 m^2) than in control subjects (11.0 ± 0.8 l/1.73 m^2, $P < 0.05$). Baseline ANP plasma values were significantly higher in IDDM patients (37 ± 4 pg/ml) than in control subjects (18 ± 5 pg/ml, $P < 0.05$). Isotonic volume expansion (saline I: 90 mmol/1.73 m^2 · 90 min; such as, 6.1 mmol/l ECV · 90 min) raised ANP plasma values to a peak concentration of 39 ± 7 pg/ml ($P < 0.01$ vs. baseline) in control subjects but not in IDDM patients (44 ± 6 pg/ml, NS vs. baseline). After the saline I load, sodium excretion rate increased significantly from 125 ± 21 μmol/min · 1.73 m^2 to 355 ± 30 μmol/min · 1.73 m^2 ($P < 0.01$) in control subjects and from 130 ± 25 μmol/min 1.73 m^2 to 207 ± 35 μmol/min/1.73 m^2 ($P < 0.05$) in IDDM. Saline II volume expansion raised significantly ANP plasma values from a baseline steady state concentration of 33 ± 6 to a peak value of 56 ± 8 pg/ml ($P < 0.01$) in IDDM patients. Sodium excretion rate went from 120 ± 31 μmol/min · 1.73 m^2 to 269 ± 43 μmol/min · 1.73 m^2.

Responsiveness to ANP (Study 3)

ANP administration resulted in similar circulating concentrations of the hormone (291 ± 35 vs. 302 ± 39 pg/ml), but resulted in significantly higher sodium excretion rates in normotensive controls (from 199 ± 14 to 340 ± 22 μmol/min · 1.73 m^2, $P < 0.01$) than in normotensive IDDM patients (from 185 ± 19 to 193 ± 21 μmol/min · 1.73 m^2, NS; Fig. 2). ANP raised significantly hematocrit and plasma protein values in normotensive control subjects but not in hypertensive IDDM patients (Fig. 2). Mean blood pressure fell and glomerular filtration rate was raised both in normotensive control and normotensive IDDM patients, albeit this hormonal action of ANP was more marked in controls than in IDDM patients (Fig. 2). Filtration fraction (FF) was increased significantly in normotensive IDDM patients but not in normotensive control subjects (Fig. 2). A resistance to the action of ANP was shown also in hypertensive IDDM patients in comparison with hypertensive control patients with regard to sodium excretion rate, hematocrit, mean blood pressure and glomerular filtration rate (Fig. 3).

Effects of angiotensin converting enzyme (ACE) inhibition in hypertensive IDDM patients (Study 4)

Three months of treatment with Cilazapril, an ACE inhibitor, significantly decreased blood pressure levels, albumin excretion rate, and baseline ANP concentration (Table 2). No change was found in GFR, whereas RPF was significantly increased. Daily sodium excretion rate increased significantly from 94 ± 14 (mmol/24 hr) to a peak value of 142 ± 18 (mmol/24 hr; $P < 0.01$) in the first month of ACE inhibitor treatment (Fig. 4). On average IDDM patients had an overall loss of 679 ± 66 mmol during the 3 month antihypertensive treatment. Thereafter daily sodium excretion rate went down to the rate shown by hypertensive IDDM patients before ACE therapy (Fig. 4). The natriuretic response to ANP challenge was improved by ACE inhibitor treatment to patterns close to those of hypertensive

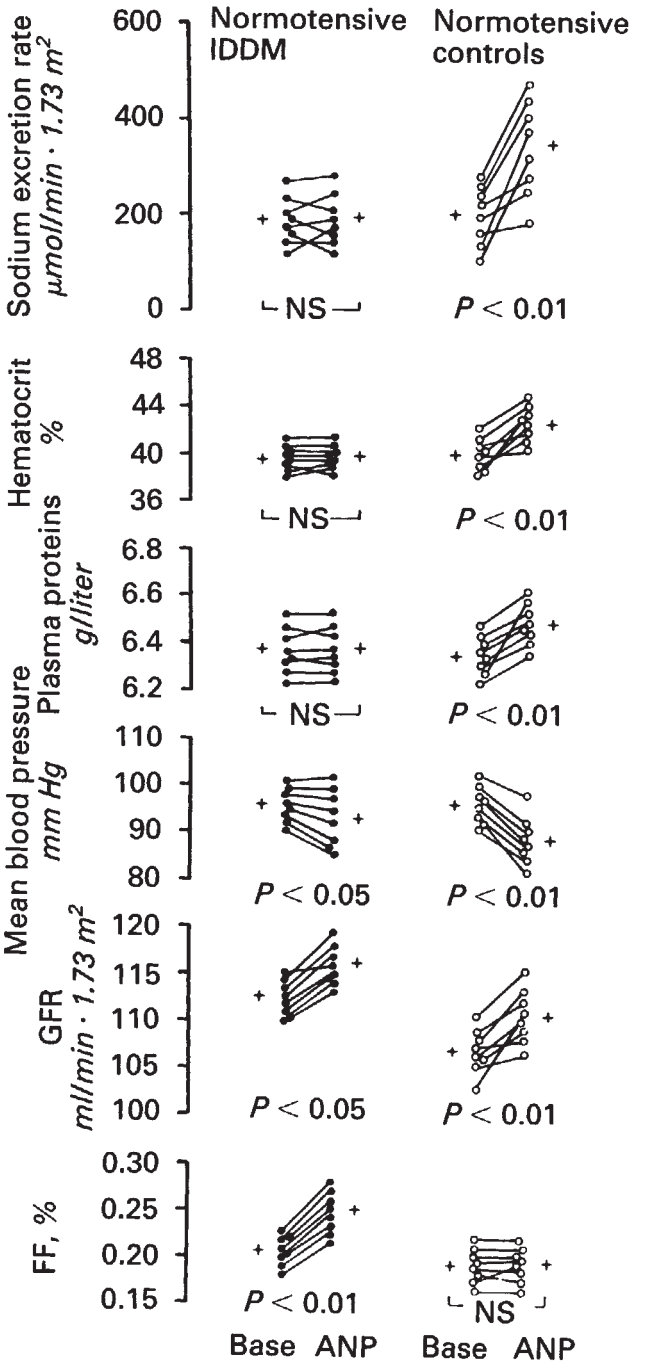

Fig. 2. *Mean ± SE and individual values of sodium excretion rate, hematocrit, plasma proteins, mean blood pressure, glomerular filtration rate (GFR) and filtration fraction (FF) in normotensive controls and IDDM patients at baseline and during ANP continuous infusion.* NS, not significant; *P* values are Base vs. ANP.

controls (from 177 ± 39 to 533 ± 42 μmol/min · 1.73 m^2, $P < 0.01$; Fig. 5). Cilazapril treatment also resulted in a significant decrease in the magnitude of ECV of hypertensive IDDM patients (from 15.98 ± 0.46 to 12.00 ± 0.45 liter/1.73 m^2, $P < 0.01$). A positive significant correlation was found between baseline ANP plasma concentrations and ECV, taking into

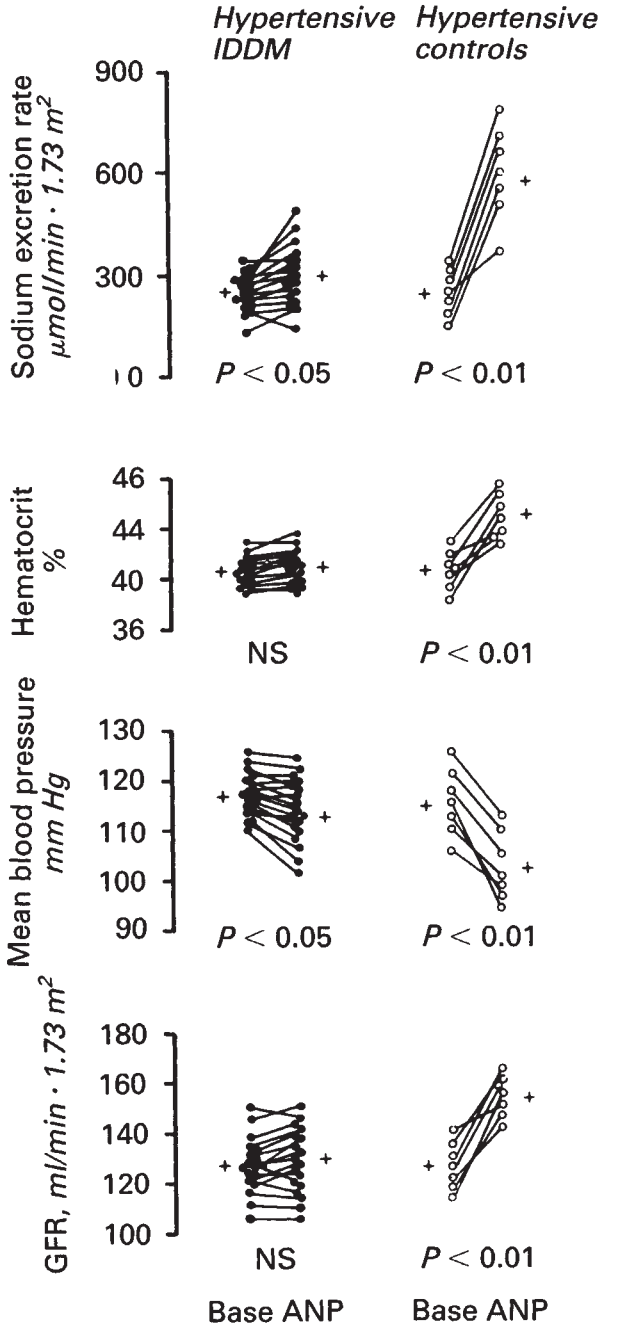

Fig. 3. *Mean ± SE and individual values of sodium excretion rate, hematocrit, mean blood pressure and glomerular filtration rate (GFR) in hypertensive controls and IDDM patients at baseline and during ANP continuous infusion.* NS, not significant; *P* values are Base vs. ANP.

consideration all the values shown by IDDM patients before and after Cilazapril treatment (r:0.79; $P < 0.01$).

Discussion

Insulin-induced sodium retention

Numerous in vitro and in vivo studies have documented that changes in plasma insulin concentration are capable of altering electrolyte transport in the kidney [22–29]. However, a limitation of these studies is that insulin administration showed an antinatriuretic action, mainly after acute administration with plasma hormone concentrations, higher than those found in normal subjects. Moreover, the subjects who participated in most of these studies followed a diet containing a supplementation of sodium chloride [26, 29]. Little information is available in humans on the sodium retentive effect of insulin after more prolonged administration which results in hormone concentrations closer to physiological levels during diets having a normal sodium intake.

With regard to insulin-dependent diabetes, it has to be pointed out that sodium excretion rate is heavily influenced by the circulating levels of glucose [30] as well as of ketone bodies, lactate and other intermediate compounds [19–21, 31, 32], which are often altered in diabetic patients with poor metabolic control with low plasma insulin levels. Therefore, to elucidate the role of insulin on sodium retention in IDDM, the sodium

Table 2. Data are mean ± SE and median with range values of blood pressure levels, albumin excretion rate, baseline ANP concentration, glomerular filtration rate and renal plasma flow in hypertensive IDDM patients before and after Cilazapril treatment

		Ace inhibitor treatment (Cilazapril)	
		Before	After
Blood pressure levels *mm Hg*	S	152 ± 8	137 ± 5[b]
	D	97 ± 3	84 ± 3[b]
Albumin excretion rate *μg/min*		18 (7–36)	10 (4–19)[b]
Glomerular filtration rate *ml/min · 1.73 m²*		128 ± 7	125 ± 8
Renal plasma flow *ml/min · 1.73 m²*		637 ± 33	661 ± 22[a]
Baseline ANP concentration *pg/ml*		38 ± 5	23 ± 7[b]

Abbreviations are: S, systolic; D, diastolic.
[a] $P < 0.05$ and [b] $P < 0.01$ before vs. after

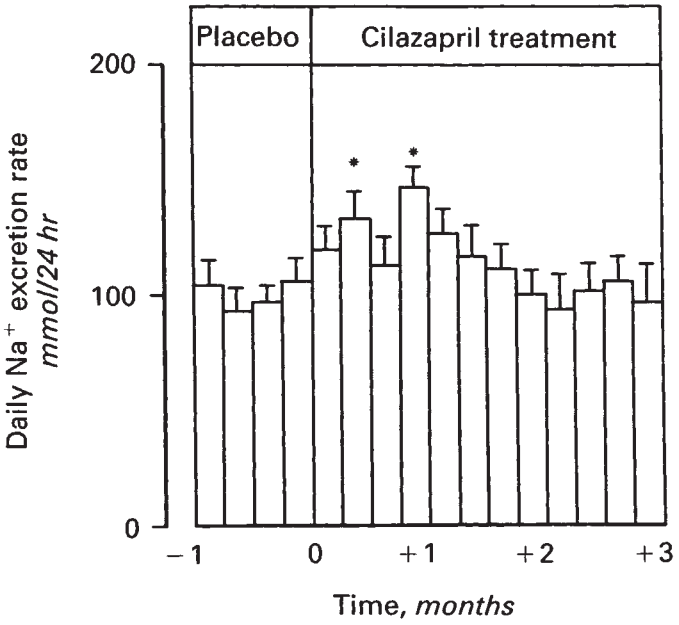

Fig. 4. *Mean ± SE daily sodium excretion rate in hypertensive IDDM patients at baseline during placebo and during 3 month ACE inhibitor (Cilazapril, Roche) treatment.* *$P < 0.05$ Cilazapril vs. Placebo.

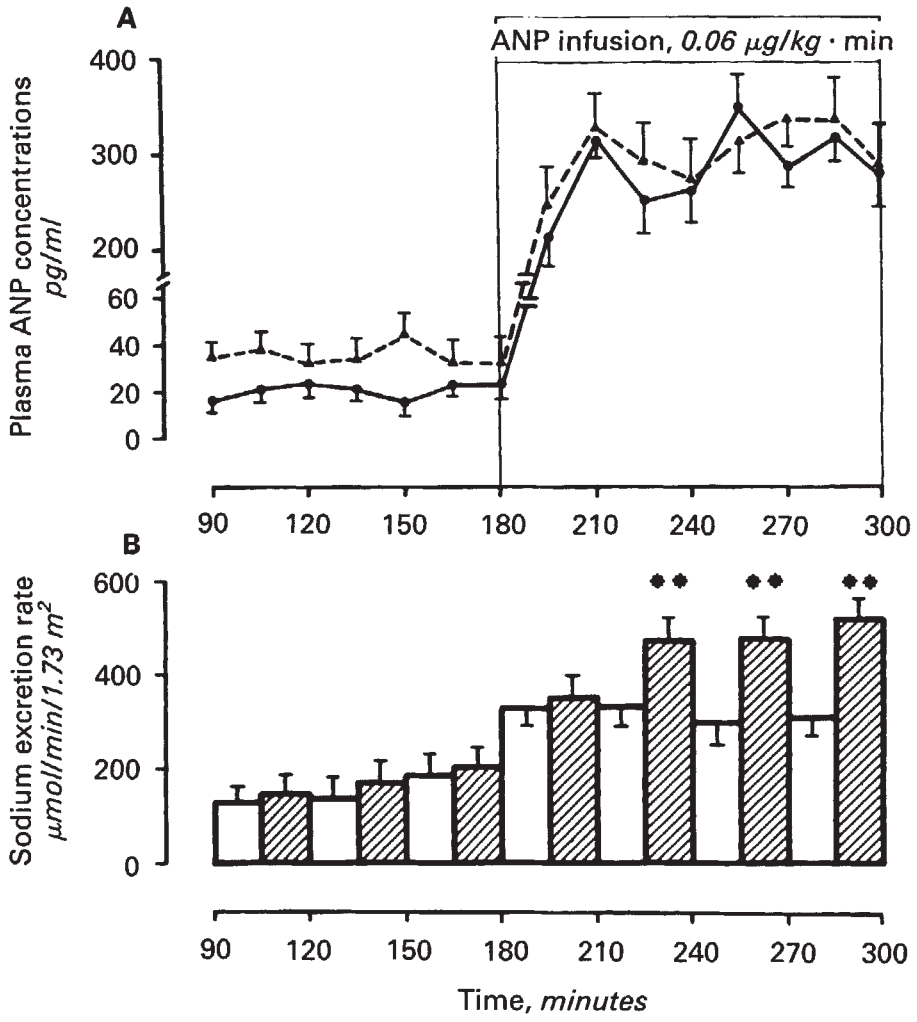

Fig. 5. *Mean ± SE plasma atrial natriuretic peptide (ANP) concentration in hypertensive IDDM patients during placebo (△ – – – △) and after 3 months of Cilazapril (● – ●) treatment at baseline and during ANP infusion (top panel).* Mean ± SE sodium excretion rate in the same patients during placebo (□) and after Cilazapril (▨) $^{**}P < 0.01$ Cilazapril vs. placebo.

excretion rate has to be investigated during hormone administration, resulting in similar insulin circulating concentrations both at hyperglycemic and euglycemic levels.

The present study demonstrates that daily sodium excretion rate is lower in euglycemic and slightly hyperinsulinemic than in hyperglycemic underinsulinized IDDM patients during a diet with normal sodium intake. Hyperinsulinemia itself, irrespective of or in addition to hyperglycemia, seems responsible for the increased transport of sodium from intratubular space to circulation, as the sodium excretion rate was significantly lower in hyperglycemic and hyperinsulinemic diabetic patients, suggesting that hyperglycemia is not capable of counterbalancing the action of insulin in renal sodium handling.

Plasma hyperinsulinemia is almost unavoidable during subcutaneous insulin therapy in IDDM, since higher circulating levels of this hormone are needed to achieve and maintain euglycemia for peripheral rather than intraportal insulin administration [17] and for insulin resistance [33]. Hyperinsulinemia, in turn, determines sodium retention and expansion of ECV. A common feature of the syndromes characterized by salt and fluid retention is the development of hormonal abnormalities in the attempt to protect the cardiovascular system from an excessive circulating volume overload [34]. In IDDM, for instance, insulin-induced sodium retention is accompanied by lower renin and aldosterone and by higher angiotensin II and norepinephrine plasma concentration, an endocrine reassessment aiming to raise kidney sodium excretion even by increasing blood pressure levels.

Thus one should predict that sodium retention and an expansion in ECV in IDDM leads to increased cardiac release of ANP [35]. ANP is indeed elevated in a subgroup of patients with essential hypertension who have low renin concentrations, a hormonal behavior resembling that of IDDM patients [36].

ANP release

The present study confirms previous findings of our laboratory [3] that euglycemic well-insulinized IDDM patients have higher baseline plasma concentrations of ANP. The most plausible mechanism accounting for this hormonal abnormality is a chronic stimulation of cardiac release into circulation of ANP, by intra- and extravascular volume expansion secondary to insulin-induced sodium retention. Our results are in keeping with those of the majority [3, 14, 37–40], but not all [2, 13, 41], of the previous reports on this matter. These discrepancies

concerning the patterns of ANP in IDDM could be due to the following reasons. First, central blood volume, rather than intravascular volume *per se,* is a determinant of ANP release. Therefore it can be postulated that ANP can be normalized whenever IDDM patients are capable of reassessing a hormonal milieu to keep a constant circulating blood volume, despite insulin-induced extracellular volume expansion. Second, most of the above-cited studies were performed in poorly-controlled IDDM patients, in whom glycosuria could have caused marked sodiuria and dehydration. Trevisan et al [42] recently showed that hyperglycemia can paradoxically ameliorate the abnormalities, both in ANP release and action, shown by IDDM patients at euglycemic levels.

With regard to ANP release in response to different stimuli, we previously found impaired ANP release during saline-induced isotonic volume expansion [3]. On the contrary, normal circulating ANP response has been found after head-out of water immersion in IDDM patients [5, 14]. These findings are only apparently in disagreement, as one has to bear in mind that IDDM patients had larger ECV. Therefore it is likely that head-down-tilt or head-out of water immersion causes a more potent redistribution of intravascular fluid volume from the periphery to the thoracic space in IDDM than in non-diabetic subjects, which in turn results in normal or even higher ANP release. On the contrary, saline infusion is not able to adequately stimulate ANP in IDDM patients, since saline load is excessively diluted in the larger ECV, which leads to impaired distension of atrial myocytes. When saline challenge is administered on the basis of the magnitude of ECV instead of body weight, ANP response to isotonic volume expansion was comparable in IDDM and control subjects. However, the sodium excretion rate was always significantly lower in IDDM than in normal subjects, whatever the infused saline load, even when the release of ANP was comparable in controls and IDDM patients. This latter finding raises the hypothesis that resistance to, rather than impaired release of ANP, plays a pivotal role in determining sodium retention in IDDM.

Resistance to ANP

The results of the present study demonstrate that the action of ANP is blunted both in normotensive and in hypertensive IDDM patients at euglycemic levels.

These findings are in agreement with the observation of Jungman et al [43] that in normotensive IDDM patients there is evidence for a decreased responsiveness to ANP with regard to excretion of renal fluids, sodium and chloride. In addition, we found that the hormonal action of ANP on kidney hemodynamics, blood pressure levels and other parameters of fluid retention, such as hematocrit and plasma proteins, was impaired in IDDM in comparison with normal subjects. At variance with the above-cited reports and with our findings, normal effects of ANP on systemic and renal hemodynamics and renal excretory function in IDDM was shown by other authors [41, 44]. Although further studies are needed to elucidate this issue, it has to be pointed out that some of these latter investigations were performed in hyperglycemic IDDM patients [41], in whom, as suggested by Trevisan et al [42], the natriuretic action of ANP could have been enhanced by hyperglycemia.

With regard to the mechanisms explaining the blunted systemic and renal response to ANP in IDDM, it is interesting that other pathologic states characterized by chronic sodium retention and ECV expansion, such as congestive heart failure [37] and experimental nephrosis [45], have been reported to have higher circulating levels and attenuated responses to ANP administration. The mechanisms underlying the abnormal renal response to ANP in IDDM are open to speculation. Among the possible hypotheses, an altered sensitivity of ANP receptors can be considered as a consequence of a decreased receptor number and/or affinity, secondary to down-regulation by elevated plasma ANP concentration. This hypothesis, however, appears unlikely, since it has been reported that rats with experimental nephrosis, in which ANP plasma concentrations are also chronically elevated, have normal ANP receptor affinity and density in renal inner and outer medulla [45].

Alternatively, the possibility can be considered that insulin can blunt the effects of ANP at the kidney level. As a matter of fact ANP action is due to a stimulation of GFR [35], to inhibition of Na^+/H^+ antiport activity in the inner medullary collecting duct cells [46], and to inhibition of Na^+/H^+ antiport in proximal tubular brush border membrane [47]. This latter mechanism is not due to the binding of ANP with specific receptors, which are missing at this kidney level, but is likely to be mediated by ANP-induced dopamine inhibition of Na^+-H^+ antiport [47]. Insulin increases sodium reabsorption by renal proximal [3, 22] and distal tubules [25, 28], and generally increases Na^+H^+ antiport activity [48].

The present study, however, provides convincing indirect evidence that the resistance to ANP action in IDDM is, to some extent, related to insulin-induced sodium retention via a chronic stimulation of ANP cardiac release by the expansion of ECV, which in turn down-regulates the hormone receptor density. The treatment with the angiotensin converting enzyme inhibitor, Cilazapril, was associated with a significant increase of sodium excretion rate in the first weeks of drug administration and with a decrease in the ECV at the end of the treatment. The overall loss of sodium pool magnitude during antihypertensive treatment was 679 mmol. This figure is roughly comparable to that calculated on the basis of the decrease in ECV (~4 liters) assuming a sodium concentration of 145 mM, such as 580 mM. The difference could be accounted for by slight changes in sodium intake after the dismissal from the ward. It is interesting to highlight that we observed similar results using diuretic therapy (data not shown), which decreases sodium pool by completely different mechanisms from ACE inhibitors. Moreover, a significant inverse correlation was found between plasma ANP baseline concentration and ECV. Interestingly, this decrease in baseline levels of ANP, was also accompanied by higher natriuretic response to hormone infusion, although the circulating levels of insulin did not change. These findings suggest that Cilazapril inhibits the chronic stimulation of ANP release, mainly by ameliorating sodium homeostasis, and that this effect is associated with an improved hormone action in vivo.

Whatever the mechanism accounting for the refractoriness to the renal and hemoconcentrating effects of ANP infusion in patients with IDDM, where endogenous plasma hormone release is chronically stimulated by insulin-induced expansion of ECV, a decreased responsiveness to, rather than an impaired cardiac release of ANP, plays a major role in the pathogenesis of sodium and water retention in IDDM and can further

deteriorate the untoward effects of insulin therapy on sodium homeostasis.

Acknowledgments

This work has been supported by CNR grant N. 9100408 PF40 e 8904006V CT04 Progretto Finalizzato Invecchiamento and by a grant of F. Hoffman-La Roche Company, Basel, Switzerland. A portion of this study was published in abstract form at the European Meeting on Hypertension, Milan, 1991 (*J Hypertens* 9 (Suppl 6): S-262, 1991).

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